in any particular progeny is frequently offset and obscured by the genetic variation among sibling seeds and those seedlings retaining undesirable or previously masked pre-cross traits.

[006] In addition to inherent genetic limitations of a traditional breeding programs, large-scale production of control pollinated seeds is also expensive. Consequently, economic and biological limitations of large-scale seed production has lead the industry to turn towards methods of asexual reproduction, such as grafting, vegetative propagation and micropropagation, as more viable alternatives.

B. Asexual (Clonal) Propagation

[007] Asexual propagation permits the application of very high selection intensity, resulting in the propagation of only those progeny showing a high genetic gain potential. These highly desirable progeny can have unique genetic combinations that result in superior growth and performance characteristics. Thus, with asexual propagation it is possible to genetically select individuals while avoiding a concomitant reduction of genetic gain due to intra-familial variation.

[008] Asexual propagation of trees can be accomplished currently by grafting, vegetative propagation, and micropropagation. Grafting, widely used to propagate select individuals in limited quantities for seed orchard establishment, is not applicable to large-scale production for reforestation. Vegetative propagation, achieved by the rooting of cuttings, and micropropagation by somatic embryogenesis, currently hold the most potential for reforestation of conifers. Although vegetative propagation by rooted cuttings can be achieved in many coniferous species, large-scale production via this method is extremely costly due to difficulties in automating and mechanizing the

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process, not to mention the need for tremendous quantities of stock tissue. This propagation method is still further limited by the fact that the rooting potential of stock plants decrease with time, making it difficult to serially propagate from select genotypes over extended periods of time.

[009] Micropropagation is the most promising method of asexual propagation for mass plantings. This process involves the production of somatic embryos *in vitro* from minute pieces of plant tissue or individual cells. The embryos are referred to as somatic because they are derived from the somatic (vegetative) tissue, rather than from the sexual process. Both vegetative propagation and micropropagation have the potential to capture all genetic gain of highly desirable genotypes. However, unlike conventional vegetative propagation methods, somatic embryogenesis is amenable to automation and mechanization, making it highly desirable for large-scale production of planting stock for reforestation. Moreover, somatic embryogenesis is particularly amenable to high intensity selection of a large number of clones. These advantages are compounded by the ability to safely preserve somatic embryogenic cultures in liquid nitrogen for long-term storage. Consequently, long-term cryogenic preservation offers immense advantages over other vegetative propagation systems that attempt to maintain the juvenility of stock plants. Techniques for somatic embryogenesis in a wide variety of plant species are well known in the art; exemplary methods for performing somatic embryogenesis in conifers are taught in U.S. Patents: 5,036,007; 5,236,841; 5,294,549; 5,413,930; 5,491,090; 5,506,136; 5,563,061; 5,677,185; 5,731,203; 5,731,204; and 5, 856,191, herein incorporated by reference in their entirety.

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[010] Thus, somatic embryogenesis has great potential for clonal production of conifer embryos to meet the increased demands of the pulp and paper industry. Assessment of embryo quality, however, needs improvement. The process of creating better tree stock begins with understanding the process of tree development from embryogenesis through full maturation.

[011] In general, plant tissue culture is the broad science of growing plant tissues on or in a nutrient medium containing minerals, sugars, vitamins and plant hormones. By adjusting the composition of the media, cultured tissues can be induced to grow or differentiate into specific cell types or organs. "Somatic embryogenesis" is a type of plant tissue culture where a piece of a donor plant is excised, cultured and induced to form multiple embryos. An embryo is a discrete mass of cells with a well-defined structure that is capable of growing into a whole plant.

[012] The methods generally in use for somatic embryogenesis today involve several steps. Prior to the tissue culture process, a suitable "explant" is harvested. A typical explant in conifer somatic embryogenesis is the "megagametophyte", a haploid nutritive tissue of the conifer seed, which is extracted from the ovule of a pollinated female cone. This ovule contains single or multiple zygotic seed embryos. In the seeds of many coniferous species, one or more genetically unique embryos naturally undergo a process called cleavage polyembryony, where a zygotic embryo grows and divides to form a small clones of embryos.

[013] The first step in somatic embryogenesis is the initiation step. The explant is placed on a suitable media. When the explant is an ovule, a process called extrusion occurs. Extrusion involves the emergence or expulsion of a zygotic embryo or multiple

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